In view of the above amendments, Applicant believes to have overcome the 35 U.S.C. § 112, second paragraph rejections.

35 U.S.C. § 102(b) Rejections – Hogan et al.

The Examiner has rejected claims 87-92 under 35 U.S.C. § 102(b) as being anticipated by Hogan et al. (U.S. Pat. No. 5,451,503) The Examiner's rejections are respectfully traversed.

Referring to Figure 15E and Examples 8-11 of U.S. Pat. No. 5,451,503, The Examiner points out that Hogan et al. teach the amplifier/anchor configuration of the present invention.

Applicant strongly believes that although Hogan et al. describe several oligonucleotide assembly configurations useful for the detection of a target nucleic acid sequence and methods of utilizing same, such configurations and methods neither anticipate nor do they render obvious the target sequence detection method described in claims 87-92 of the instant application.

Although Hogan et al., in one embodiment (example 8, column 21) of the referenced prior art disclose the cleavage of "...an active restriction enzyme site removed from the target strand, ...the cleavage site designed to be close to the base of the arm region duplex" such that "...upon cleavage the arm regions are ...removed from the 3-way branched...structure, reducing the stability of the overall structure", this mechanism of detection of hybridization between probes and target sequences is dependent on the melting of the probes from the target, requiring repetition of the entire hybridization and cleavage process for amplification of detection signal.

In fact, Hogan et al. repeatedly emphasize that "once cleavage occurs the probe regions will melt off of the target. This will then allow uncleaved probe strands to hybridize with the target and start the process over again, thus increasing assay sensitivity by cycling multiple probes through a single target site" (column 21 lines 38-42).

Thus, contrary to the assertions of the Examiner, Hogan et al. do not describe nor suggest oligonucleotide-target cycling (recycling). Figure 15E of Hogan et al. does not describe a probe configuration which is analogous to the probe configuration of the present invention, but rather, Figure 15E illustrates an RNA-DNA probe, a portion of which (an arm of the RNA oligonucleotide) can be degraded using RNAse H. Following degradation both oligonucleotides are melted off the target and separated using PAGE (to allow detection). In the next step, another RNA-DNA probe is hybridized with the target (cycling).

In sharp distinction from Hogan et al., the oligonucleotides of claims 87-92 are designed with the intention of decreasing the orders of the hybridization and dissociation (following cleavage) reactions between the oligonucleotide and the target nucleic acid sequence, since such a decrease in reaction order results in an increase in signal amplification.

To decrease the reaction order, the inventors have designed one oligonucleotide ("anchor") such that it stably hybridizes with the target nucleic acid sequence under the reaction conditions used. Such stable hybridization between the anchor oligonucleotide and target can be provided, for example, by a target complementary sequence (first region of the anchor oligonucleotide) of a Tm substantially higher that the reaction temperature.

The second oligonucleotide of the system (the "amplifier") is designed such that it hybridizes with the target nucleic acid sequence substantially only in the presence of a hybridized anchor oligonucleotide thus increasing hybridization efficiency and decreasing the likelihood of non-specific hybridization. Following hybridization of the amplifier oligonucleotide with the target-anchor, a nucleic acid cleavage site is formed which is nuclease-cleavable only in the amplifier sequence ("second region" of the amplifier oligonucleotide). Whereas the oligonucleotide system disclosed by Hogan et al. forms a nucleic acid cleavage site comprising two discontinuous

complementary strands, the "duplex structure" including a nucleic acid cleavage site formed by the oligonucleotides of the method of the instant application is a result of the presence of a looped structure (see Figure 1 of the instant application) of the amplifier, and the short complementary sequence of the anchor oligonucleotide. This constitutes a uniquely stable structure when hybridized, since the loop and nick inherent in the (amplifier-anchor) duplex structure (see Figure 1 of the instant application) produce stabilizing stem-stacking forces. Cleavage of the duplex structure of the present invention destabilizes the amplifier oligonucleotide and causes its release from the target, while the anchor oligonucleotide remains hybridized to the target ("oligonucleotide-target nucleic acid sequence hybrid") and as such is recycled along with it and used in each of the subsequent self-cycling steps of the reaction.

It will be appreciated that in addition to decreasing reaction order, the use of an anchor oligonucleotide of the present invention enables minimizing the quantity (copy number) of such an oligonucleotide used in the reaction, thus minimizing or eliminating background signals associated with non-specific hybridization resultant from the presence of larger oligonucleotide quantities. Furthermore, hybridization of the anchor oligonucleotide to the target provides the target sequence with additional chain length and volume, thereby increasing the available interface area for the subsequent hybridization with the amplifier oligonucleotide. Such an increase in hybridization interface area results in both increased efficiency and specificity of hybridization with the amplifier.

Such probe design is neither described nor suggested in U.S. Pat. No. 5,451,503. Although Hogan et al. describe degradation of one oligonucleotide region (Figure 15E), such degradation will not lead to oligonucleotide-target recycling, since oligonucleotide-target recycling requires selective melting off of one oligonucleotide (amplifier) which can be achieved, for example, by

selecting different melting temperature for each oligonucleotide of the probe. Since Hogan et al. do not describe selective melting, or oligonucleotide-target recycling, following RNAse H degradation, both annealed strands will melt off the target sequence.

Thus, upon careful review of U.S. Pat. No. 5,451,503, and while considering the arguments raised by the Examiner, Applicant still maintains the opinion that the method of detection of claims 87-92 is neither described or suggested by Hogan et al.

In view of the above amendments and remarks it is respectfully submitted that claims 87-92 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

A three month extension fee had previously been authorized and charged to Deposit Account 50-1407 on January 7, 2003. In the event additional fees are necessary for this response, authorization to charge the above Deposit Account is hereby authorized.

Respectfully submitted,

Sol Sheinbein
Attorney for Applicant
Registration No. 25,457

Date: January 21, 2003.

Encl.:
VERSION WITH MARKINGS TO SHOW CHANGES MADE

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

87. (Amended) A method of detecting a presence or an absence of a target nucleic acid sequence in a sample, the method comprising the steps of:

(a) contacting the sample with an oligonucleotide system under hybridization conditions so as to form a reaction mixture, said oligonucleotide system including an anchor oligonucleotide and an amplifier oligonucleotide, each of said anchor and said amplifier oligonucleotides including a first region ~~being capable of hybridizing~~complementary with the target nucleic acid sequence, each of said anchor and said amplifier oligonucleotides further including a second region, said second regions of said anchor and said amplifier oligonucleotides being at least partially complementary and thus capable of forming a duplex structure including a nucleic acid cleaving agent recognition sequence following hybridization of said first regions of said anchor and said amplifier oligonucleotides with the target nucleic acid sequence, said anchor and said amplifier oligonucleotides are selected such that when hybridized with the target nucleic acid sequence in a presence of a nucleic acid cleaving agent recognizing said nucleic acid cleaving agent recognition sequence, only said amplifier oligonucleotide is cleavable by said nucleic acid cleaving agent, wherein cleavage of said amplifier oligonucleotide leads to dissociation of said amplifier oligonucleotide from the target nucleic acid sequence while said anchor oligonucleotide remains hybridized to the target nucleic acid sequence to form a stabilized anchor oligonucleotide-target nucleic acid sequence hybrid thereby allowing a second and

uncleaved amplifier oligonucleotide to hybridize with said anchor oligonucleotide-target nucleic acid sequence hybrid thus enabling recycling of said anchor oligonucleotide-target nucleic acid sequence hybrid with respect to said amplifier oligonucleotide.

(b) adding said nucleic acid cleaving agent to said reaction mixture under predetermined reaction conditions, such that, if the target nucleic acid sequence is present in the sample, said nucleic acid cleaving agent recognition sequence is cleaved by said nucleic acid cleaving agent; and

(c) monitoring cleavage of said nucleic acid cleaving agent recognition sequence by said nucleic acid cleaving agent;

wherein cleavage of said nucleic acid cleaving agent recognition sequence by said nucleic acid cleaving agent indicates hybridization of the oligonucleotide system to the target nucleic acid sequence and therefore the presence of the target nucleic acid in the sample.